Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Insect Physiology 52 (2006) 442–449
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Gas exchange characteristics, metabolic rate and water loss of the Heelwalker, *Karoophasma biedouwensis* (Mantophasmatodea: Austrophasmatidae)

S.L. Chown[a,*], E. Marais[a], M.D. Picker[b], J.S. Terblanche[a]

[a]*DST-NRF Centre for Invasion Biology, Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*
[b]*Department of Zoology, University of Cape Town, Rondebosch 7700, South Africa*

Received 4 August 2005; received in revised form 16 December 2005; accepted 20 December 2005

Abstract

This study presents the first physiological information for a member of the wingless Mantophasmatodea, or Heelwalkers. This species shows cyclic gas exchange with no evidence of a Flutter period (more typical of discontinuous gas exchange in insects) and no indication that the spiracles are fully occluded during quiescent metabolism. Standard metabolic rate at 20 °C was 21.32 ± 2.73 μl $CO_2\,h^{-1}$ (mean ± S.E.), with a Q_{10} (10–25 °C) of 1.7. Increases in $V^{\bullet}CO_2$ associated with variation in mass and with trial temperature were modulated by an increase in burst period volume and a decline in cycle frequency. Total water loss rate, determined by infrared gas analysis, was 0.876 ± 0.08 mg $H_2O\,h^{-1}$ (range 0.602–1.577, $n = 11$) whilst cuticular water loss rate, estimated by linear regression of total water loss rate and metabolic rate, was 0.618 ± 0.09 mg $H_2O\,h^{-1}$ (range 0.341–1.363, $n = 11$). Respiratory water loss rate was therefore no more than 29% of the total rate of water loss. Both total water loss rate and estimated cuticular water loss rate were significantly repeatable, with intraclass correlation coefficients of 0.745 and 0.553, respectively.
© 2006 Elsevier Ltd. All rights reserved.

Keywords: Gas exchange; Mantophasmatodea; Metabolic rate; Repeatability; Respiratory water loss

1. Introduction

The Mantophasmatodea is best known as the most recently described insect order. Apart from an old Tanzanian species, extant members of the order are restricted to the south-western parts of Southern Africa. Although fossil Baltic amber specimens had been described previously (Zompro, 2001), the order was only recognized in 2002 (Klass et al., 2002), and old material was soon unearthed in collections of several South African museums (Picker et al., 2002). Subsequent work on the new order has largely been concerned with systematics, description of new fossil species, and taxonomy and morphology (Klass, 2002; Picker et al., 2002; Tilgner, 2002; Zompro et al., 2002, 2003; Dallai et al., 2003; Klass et al., 2003; Walker, 2003; Engel and Grimaldi, 2004; Gullan and Cranston, 2005; Grimaldi and Engel, 2005). Some work has been published on their embryology and reproduction (Machida et al., 2004; Tojo et al., 2004; Tsutsumi et al., 2004) and peptides (Gäde et al., 2005; Predel et al., 2005). A sister group relationship with Ice-crawlers (Grylloblattodea) has been postulated based on DNA sequencing and some morphology (Terry and Whiting, 2005), although Dallai et al. (2003) suggested a relationship with Mantodea based on sperm morphology.

What is presently known of the order is that its extant members are restricted to semi-arid regions of Namibia and south-western South Africa, with the exception of a single record from Tanzania (Klass et al., 2002). The monophyletic group of secondarily flightless insects presently includes 13 extant-described species (Klass et al., 2003; Zompro et al., 2003), several extant undescribed ones, and at least three species noted from Baltic amber (Zompro et al., 2002; Engel and Grimaldi, 2004). The extant species are found primarily in rocky, arid habitats in Namibia, and

*Corresponding author. Tel.: +27 21 808 2385; fax: +27 21 808 2995.
E-mail address: slchown@sun.ac.za (S.L. Chown).

0022-1910/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2005.12.004

in the base of tussock vegetation and in low shrubs in the Western and Northern Cape Provinces of South Africa. Adults of the South African species are nocturnal. They show pronounced sexual size dimorphism (smaller males), and are carnivorous, feeding on a variety of small insects and other arthropods (Picker et al., 2002; Zompro et al., 2002, 2003; Walker, 2003). In South Africa the nymphs hatch after the first winter rains, and complete their development during the wet and fairly cold winter, reaching maturity in spring, when the soils are still moist, and there is an abundance of insect life and flowers. Adults die at the onset of summer, leaving diapausing eggs in a pod in the soil (Tojo et al., 2004).

Little is known of the physiology of the members of the new order. By comparison, most other insect orders have enjoyed considerable study of at least one, but often many more than one of their constituent species, from a broad range of physiological perspectives (Chapman, 1998; Chown et al., 2002; Nation, 2002; Chown and Nicolson, 2004). However, for particular physiological characteristics the lack of attention given to the group is not especially remarkable (see Chown et al., 2002), but is nonetheless problematic for large-scale comparative analyses (Chown et al., 2004a, b). Hence, a key goal of comparative analyses, especially at the macrophysiological scale, must be the representation of as wide a range of taxa and sites as is necessary to validate the generality of previous conclusions that have been based on a narrower data set. Here we contribute to this goal by providing information on gas exchange characteristics, metabolic rates and water loss of *Karoophasma biedouwensis* (Klass et al., 2003). In doing so we broaden the comparative data base for these characteristics to another order of insects, the Mantophasmatodea.

2. Materials and methods

Individuals of *K. biedouwensis* were collected by sweeping and beating vegetation at the farm Wolfdrif, Pakhuispad, Clanwilliam district, Western Cape Province, South Africa (site grid reference: 30°00′S, 19°05′E) or hand collected from the bases of individual tussock-like Restionaceae plants over several days at the end of August 2004. The insects rest within the tightly packed culms at the base of the restio by day. One of these plants (*Wildenowia* sp.) was instrumented with five Hobo Pro (Onset Computing, Pocasset, MA) dataloggers that recorded temperature ($n = 5$) and relative humidity ($n = 4$), measured using internal probes, over 4 days (including the collection date). These loggers were placed within the restio tussock at its base (Base), within the tussock but suspended 50 cm above the ground (Core), about 1 m outside the tussock on the ground on its southern side (South), and in a similar position but to the north of the tussock (North). The loggers were set to record temperature and humidity every 5 min. Means, minima and maxima for both temperature and relative humidity for the full period, and for day and night values (sunset was taken as 18h26 and sunrise as 07h01 based on the tables obtained from http://www.aa.usno.navy.mil/cgi-bin/aa_rstablew.pl) were calculated, but formal comparisons between logger sites were not made because of temporal autocorrelation in the data.

The insects were returned to the laboratory within 2 days of collection. Here they were held in glass vials, with access to water, in a climate-controlled chamber at 22 ± 1.5 °C (12L: 12D). Individuals were fed either 3–4 vinegar flies (*Drosophila* sp.) or 1–2 fruit flies (*Ceratitis* sp.) every day immediately after respirometry had been completed to ensure that they did not starve. Typically, the individuals consumed the food immediately, and were then starved for >12 h prior to the next respirometry trial if they were used in another trial (see below for a description of repeatability assessments at 20 °C). Preliminary experiments, addressing how long the effects of specific dynamic action (SDA) lasted, showed that >12 h was a reasonable compromise between likely metabolic down regulation as a consequence of starvation (see Lighton, 1989), and metabolic elevation as a consequence of SDA. Trials were undertaken at 10, 20 and 25 °C daily between 08h00 and 18h00. For each trial, an individual was weighed (to 0.1 mg on a Mettler AX-504 electronic balance), and placed into a darkened 5 ml cuvette. Individuals were allowed to settle in the cuvettes for approximately 10 min prior to commencing the recording. Bottled air was then pushed via a Side-trak mass flow controller at a rate of 50 ml min^{-1} through soda lime, silica gel and Drierite columns to scrub it of residual CO_2 and H_2O, over the individual in the cuvette, and into the detector cells of either a LiCor 7000 (LiCor, Lincoln, Nebraska) or LiCor 6262 oxygen analyzer, that had been calibrated using a CO_2 standard and a LiCor 610 vapor pressure generator. The output of the analyzers ($V^{\bullet}CO_2$ and $V^{\bullet}H_2O$) was stored on standard desktop pc either via DATACAN V (Sable Systems, Henderson, Nevada) in the case of the Li-6262, or via LiCor software (Li-7000). Data for each individual were accompanied by baseline readings for the empty cuvette, both before and after the trial, and by information from an auxiliary channel on the activity pattern of the individual, derived from Sable Systems infrared AD-1 activity detectors (see www.sablesystems.com). Following a trial, which generally lasted for 2 h, individuals were weighed again and the mean mass for the trial was used in all further calculations.

The data for each individual were subsequently imported into DATACAN V (via Microsoft Excel in the case of the Li7000), corrected to standard temperature and pressure, and analyzed initially using the customized functions of this software. Only those sections of gas exchange traces that corresponded to periods of zero activity of the individuals were analyzed. Although individuals regularly showed periodic gas exchange, they also showed continuous gas exchange that was not associated with activity. For the gas exchange (i.e. CO_2 release) characteristics, only information from individuals, which showed cyclic gas exchange traces, was used. For each individual, a minimum of three cycles was used for determination of CO_2 release

characteristics (mean $V^{\bullet}CO_2$ ($\mu l\,h^{-1}$), cycle frequency (mHz), burst volume (μl), duration (h), and rate ($\mu l\,h^{-1}$), and interburst volume (μl), duration (h) and rate ($\mu l\,h^{-1}$)), and the means of these values formed the individual data that were used for statistical analyses. The interburst period was defined as the period between the lowest value at the end of a burst and the corresponding value prior to the following burst. Burst periods were those periods between interburst periods. Summary statistics for each of the variables were obtained using Statistica version 6.0 (Statsoft, Oklohoma). Because none of the variables were normally distributed, they were $\log_{10}$-transformed prior to further analyses. The relationships between these variables and trial temperature were investigated using ordinary least squares regression (implemented in Statistica). General linear models were used to investigate the extent to which variation in mean $V^{\bullet}CO_2$ ($\mu l\,h^{-1}$) could be explained by variation in temperature, mass and sex, and by variation in the gas exchange characteristics (see Lighton, 1991; Davis et al., 1999).

For investigations of water loss rate, data from the water channel of the infrared gas analyzers were used from the 20 °C trials, having been processed as above (i.e. adjusted for the baseline of the empty cuvette and corrected to standard temperature and pressure), and verified against a gravimetric estimate of water loss. For each individual, we expected gravimetric water loss to be larger than that determined using the gas analyzers because the traces we analyzed were for periods of inactivity and not for the full period of recording. This was indeed the case (see Results), and so we place less emphasis on the gravimetric water estimates of water loss here, and also because they also include a small amount of mass loss due to metabolism. The water and CO_2 data were then analyzed following the methods of Gibbs and Johnson (2004) to estimate cuticular and respiratory water loss. This method uses an ordinary least squares regression of $V^{\bullet}H_2O$ (dependent variable) on $V^{\bullet}CO_2$ (independent variable) for each individual. The *y*-intercept then provides an estimate of cuticular water loss rate because no water can emerge from the spiracles if gas exchange is not taking place (i.e. the spiracles are theoretically closed). The difference between total water loss rate and cuticular water loss rate provides an estimate of respiratory water loss rate. This technique makes the assumption that in insects that do not fully close their spiracles the regression line can be extrapolated to a *y*-intercept of zero. Clearly this is a questionable statistical assumption (linearity beyond the available data cannot be assumed). However, in the absence of other non-manipulative methods for calculating cuticular and spiracular water loss rates in animals that do not close their spiracles, it is one that must be made (see Lighton et al., 2004 for a manipulative method). Since this method should be applicable to several gas exchange patterns (Gibbs and Johnson, 2004), we added two additional individuals that did not show clear cyclic $V^{\bullet}CO_2$ traces (i.e. were excluded from the summary statistics presented in Table 1). Thus, for these analyses six individuals for which a single trace was obtained were included, while five individuals for which repeated measures were available were also included (i.e. $n = 11$). Because we were concerned about the assumptions of the approach adopted by Gibbs and Johnson (2004), we also determined the extent to which the cuticular water loss rate estimates, based on the method, are repeatable, and did the same for the total water loss rates estimated by analyses of the water channel traces from the IRGAs. Repeatability was assessed by obtaining four repeated measurements for each of five individuals at 20 °C (although repeated measures finally used for the analyses ranged from 2 to 4 within individuals, individuals $n = 5$). Total water loss rates and the cuticular water loss were then calculated for each repeated measure. Thereafter, the repeatability of each of the parameters was determined by calculating *r* (the intraclass correlation coefficient), based on analyses of variance (Lessells and Boag, 1987), and its 95% confidence limits, using the formulae provided by Krebs (1999).

Table 1
Means ± S.E. for $V^{\bullet}CO_2$ and cycle frequency, for interburst period emission rate, volume and duration, and for burst period emission rate, volume and duration in *Karoophasma biedouwensis*

Variable	10 °C ($n = 7$)	20 °C ($n = 9$)	25 °C ($n = 11$)
Mass (mg)	84.65 ± 17.56	103.99 ± 31.78	94.03 ± 14.70
$V^{\bullet}CO_2$ ($\mu l\,h^{-1}$)	16.68 ± 5.03	21.32 ± 2.73	32.12 ± 4.02
Frequency (mHz)	5.11 ± 1.29	3.22 ± 0.31	3.83 ± 0.26
Interburst $V^{\bullet}CO_2$ ($\mu l\,h^{-1}$)	10.53 ± 3.92	18.44 ± 5.83	21.43 ± 2.98
Interburst volume (μl)	0.066 ± 0.019	0.662 ± 0.221	0.500 ± 0.099
Interburst duration (h)	0.013 ± 0.006	0.037 ± 0.006	0.026 ± 0.006
Burst $V^{\bullet}CO_2$ ($\mu l\,h^{-1}$)	17.83 ± 5.16	25.74 ± 2.91	39.04 ± 6.69
Burst volume (μl)	0.822 ± 0.092	1.541 ± 0.264	2.054 ± 0.439
Burst duration (h)	0.064 ± 0.013	0.059 ± 0.006	0.051 ± 0.003

3. Results

The collection site was characterized by variable, but generally mild to warm temperatures, with the *Wildenowia* sp. core and base temperatures tending to be intermediate between the warm northern and cool southern sides of the plant (Fig. 1a). The relative humidity within the plants and in its surrounding environment hardly differed, at least at this scale of assessment (Fig. 1b).

Individual *K. biedouwensis* showed both continuous and periodic gas exchange during rest (Figs. 2 and 3), although three individuals showed continuous gas exchange only. The remaining individuals all showed cyclic gas exchange in one or more trials, and this cyclic gas exchange was typified by clear burst and interburst periods, but not by distinct Open, Flutter and Closed periods which are typical of discontinuous gas exchange cycles (Lighton, 1996). Indeed, it appears from the $V^{\bullet}CO_2$ traces that the spiracles in *K. biedouwensis* rarely close fully (Fig. 3; see also Table 1) or if they do, closure was too quick to be detected

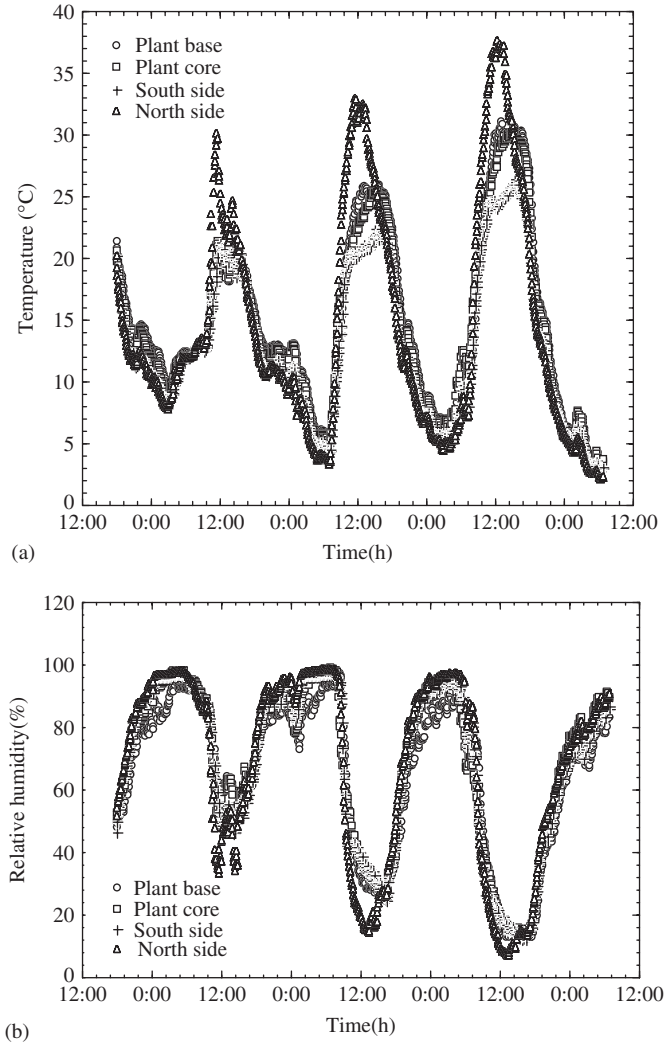

Fig. 1. Variation in (a) temperature and (b) relative humidity, over 4 days in late August 2004 within (plant base and core) and next to (northern and southern aspects) a restio plant (*Wildenowia* sp.) that was occupied by several *Karoophasma biedouwensis* individuals.

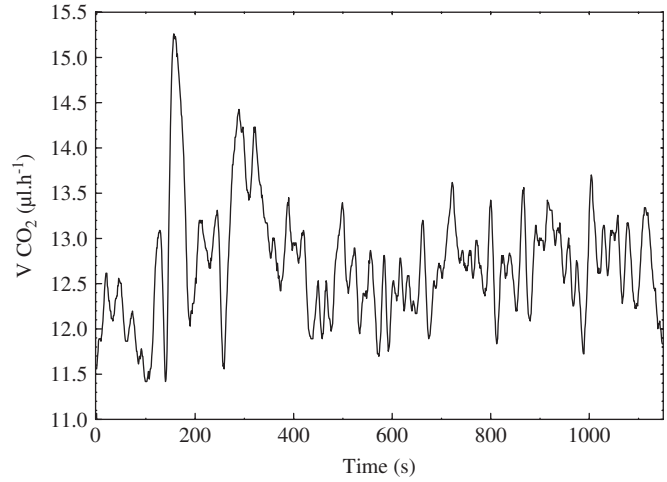

Fig. 2. Continuous gas exchange in a *K. biedouwensis* at rest at 20 °C (mass = 39.45 mg).

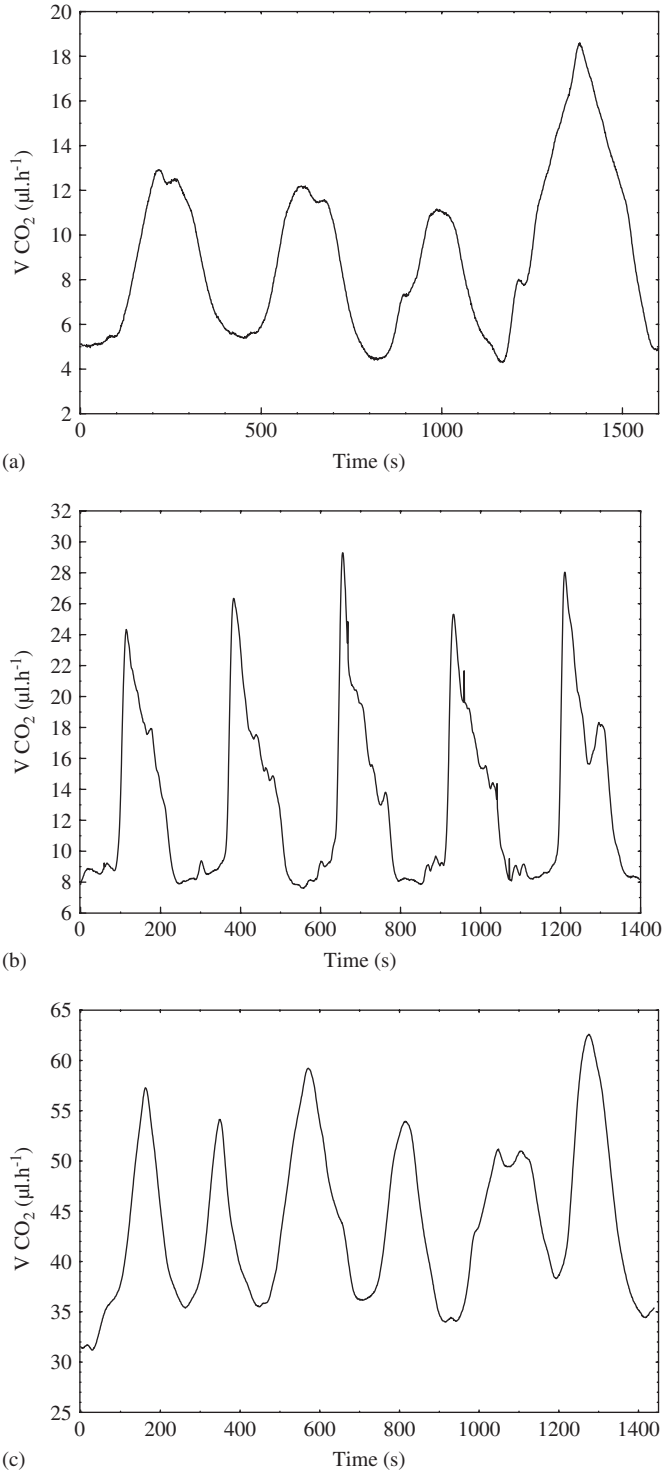

Fig. 3. Sample $\dot{V}CO_2$ traces for *K. biedouwensis* at: (a) 10 °C (mass = 57.0 mg), (b) 20 °C (mass = 145.1 mg), and (c) 25 °C (mass = 144.6 mg).

by our experimental design in which sampling took place at 1 s intervals.

Increasing trial temperature resulted in significant increases in metabolic rate, and several of the other gas exchange characteristics varied significantly and positively with temperature (Tables 1 and 2). In the general linear

Table 2
Ordinary least squares regression relationships between temperature and the logged values of $V^{\bullet}CO_2$, cycle frequency, interburst period emission rate, volume and duration, and burst period emission rate, volume and duration in *Karoophasma biedouwensis* (degrees of freedom = 1, 25 in all cases)

Variable	Intercept ± S.E.	Slope ± S.E.	R^2	F	p
$V^{\bullet}CO_2$ (μl h^{-1})	0.881 ± 0.140	0.023 ± 0.007	0.277	10.97	0.002
Frequency (mHz)	—	—	—	0.56	0.46
Interburst $V^{\bullet}CO_2$ (μl h^{-1})	0.635 ± 0.165	0.026 ± 0.008	0.269	10.56	0.003
Interburst volume (μl)	−1.847 ± 0.235	0.064 ± 0.012	0.537	31.19	0.0001
Interburst duration (h)	−2.467 ± 0.261	0.037 ± 0.013	0.223	8.45	0.008
Burst $V^{\bullet}CO_2$ (μl h^{-1})	0.911 ± 0.134	0.025 ± 0.007	0.338	14.30	0.009
Burst volume (μl)	−0.334 ± 0.139	0.023 ± 0.007	0.286	11.43	0.002
Burst duration (h)	—	—	—	0.12	0.73

Table 3
Results of general linear models exploring the relationship between variation in $V^{\bullet}CO_2$ and variation in (a) mass, temperature and sex; and (b) mass, temperature, sex, interburst (IB) emission volume, interburst duration, burst (B) emission volume and burst duration in *Karoophasma biedouwensis*

Variable	df	MS	F	P	Estimate
(a)					
Intercept	1	0.00057	0.033	0.857	
Temperature	1	0.513	29.49	0.0001	0.0229
Log mass	1	0.180	10.36	0.004	0.491
Sex	1	0.040	2.29	0.143	
Error	25	0.0174			
(b)					
Intercept	1	0.0128	1.809	0.193	
Temperature	1	0.0185	2.621	0.120	
Log mass	1	0.0127	1.796	0.195	
Log IB volume	1	0.0237	3.353	0.081	
Log IB duration	1	0.0421	5.959	0.024	−0.315
Log B volume	1	0.0627	8.879	0.007	0.476
Log B duration	1	0.0487	6.890	0.016	−0.513
Sex	1	0.0015	0.206	0.655	
Error	21	0.0071			

MS, mean squares; df, degrees of freedom; F, F-ratio.

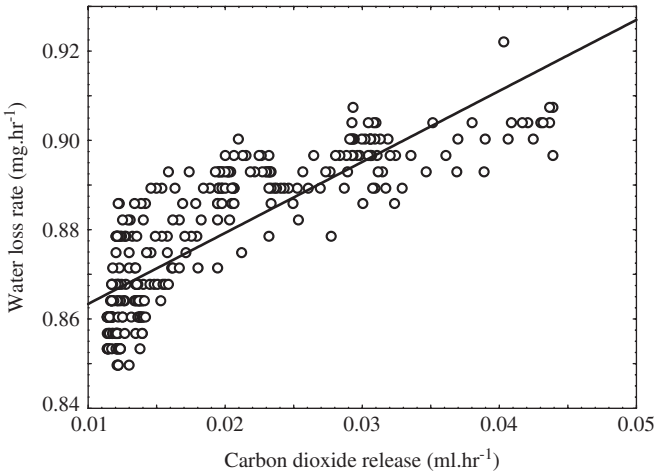

Fig. 4. Plot of water loss versus CO_2 release for a single *K. biedouwensis* male measured at 20 °C during a period of cyclic gas exchange (flow rate = 50 ml min^{-1}; body mass = 0.0436 g). Equation for the linear regression: $y = 1.5904x + 0.8475$ ($r^2 = 0.681$).

model including mass, temperature and sex as the independent variables it was clear that mass and temperature accounted for most of the variation in $\log_{10} V^{\bullet}CO_2$ (Table 3). Although sex on its own was a significant variable (analysis not shown), this was likely a function of sex-related mass variation (females (128.6 ± 7.6 mg, mean ± S.E.) are much larger than males (54.9 ± 8.3 mg)), and is reflected entirely in the mass component of the general linear model, making the sex term non-significant. When the analysis was repeated excluding sex, the coefficient for mass increased to 0.658, the 95% confidence intervals of which (0.436–0.881) included both of the traditional scaling exponents of 0.67 and 0.75. When interburst and burst emission volumes and durations were included in the model, the mass and temperature variables were non-significant and the former (excluding interburst volume) explained 88% of the variation in $\log_{10} V^{\bullet}CO_2$. In particular, increases in metabolic rate were accompanied by an increase in burst volume and a decline in both interburst and burst durations (Table 3).

Total water loss rate at 20 °C measured using the gravimetric method was 1.244 ± 0.103 mg H_2O h^{-1} (range 0.900–2.073, $n = 11$) and using the gas analysis method was 0.876 ± 0.08 mg H_2O h^{-1} (range 0.602–1.577, $n = 11$). The gravimetric estimate was significantly larger than the gas analysis one (ANOVA $F_{(1,20)} = 7.93$, $p = 0.011$), which is unsurprising given handling time, extra time spent in the cuvette, and the small contribution of metabolism to mass loss. Estimated cuticular water loss rate was 0.618 ± 0.09 mg H_2O h^{-1} (range 0.341–1.363, $n = 11$) (see Fig. 4). The latter value includes water that might have been lost through other avenues such as the mouth and anus. Across the 11 individuals, the relationship between cuticular water loss rate and total water loss rate was positive and significant (total water loss = 0.814 (S.E. = 0.150) × cuticular water loss + 0.373 (S.E. = 0.101); $r^2 = 0.74$; $F_{(1,9)} = 29.39$, $p < 0.0005$, SE of estimate = 0.136). Although the expression of respiratory water loss rate as a percentage of the total water loss rate can lead to misinterpretation (Chown, 2002), it is nonetheless

Table 4
Outcome of the analyses of variance and calculations of repeatability for total water loss rate and estimated cuticular water loss rate for *Karoophasma biedouwensis*

Parameter	Effect	SS	DF	MS	*F*-ratio	*p*	Repeatability
Total water loss rate	Between-individual	1.817	4	0.454	10.899	< 0.001	0.745
	Within-individual	0.500	12	0.042			(0.965, 0.326)
Cuticular water loss rate	Between-individual	1.003	4	0.251	5.151	0.012	0.553
	Within-individual	0.584	12	0.049			(0.929, 0.070)

The confidence limits for the repeatability values are indicated in parentheses.

commonly done. In this case, respiratory water loss rate accounted for no more than 29% of the total water loss rate, and probably somewhat less given that small amount of water was probably lost via other routes. Repeatability of both total water loss rate and estimated cuticular water loss rate were significant and high (Table 4). Although the repeatability of the latter was much lower than the former, their 95% confidence limits overlapped substantially.

4. Discussion

Gas exchange in *K. biedouwensis* is cyclic, but it does not take the typically discontinuous gas exchange cycle form of the kind seen in many lepidopteran pupae, beetles and ants (reviewed in Lighton, 1996, 1998). Rather, there is no discernible *F*-period, and the spiracles do not close fully (at least not so far as we could ascertain), making this species similar to other Polyneoptera, such as termites and some cockroaches, which also show cyclic, but not discontinuous, gas exchange (Shelton and Appel, 2000, 2001; Marais and Chown, 2003). Indeed, Marais et al. (2005) have shown that cyclic gas exchange, with no *F*-period and incomplete spiracular occlusion, is basal within the insects and typical of most apterygotes and Polyneoptera. Cyclic gas exchange is therefore not unexpected in the Mantophasmatodea, which, together with the Grylloblattodea, forms the sister-group to the clade comprising Isoptera, Mantodea and Blattodea (Terry and Whiting, 2005).

The positive effects of temperature on metabolic rate, and a Q_{10} value of 1.7 (derived from the slope of the rate–temperature relationship) are not surprising because both are typical of insects (Keister and Buck, 1964; Chown and Nicolson, 2004). The mass scaling exponent (or coefficient) for metabolic rate included both 0.67 and 0.75, which have variously been argued to have sound underlying mechanistic explanations (see Schmidt-Nielsen, 1984; West et al., 1999; Dodds et al., 2001). That the scaling exponent found here should be close to 0.75 is predicted both by the West et al. (1999) fractal geometry model and the alternative model proposed by Kozłowski et al. (2003), and does not provide a ready means to distinguish between them especially because sample sizes here were relatively low. However, the considerable size variation within *K. biedouwensis* may prove to be useful for further exploring whether the intraspecific mass scaling exponent is the same as the interspecific scaling exponent, an explicit prediction of the fractal model (West et al., 2002), but not necessarily of the cell size model (Kozłowski et al., 2003).

Changes in $V^{\bullet}CO_2$, associated with variation in temperature and in mass, were modulated largely by an increase in burst volume and a decline in the duration of both the burst and interburst periods ($\approx$increase in cycle frequency). The increase in cycle frequency shown by *K. biedouwensis* is similar to that found in many other species which show discontinuous or cyclic gas exchange, where temperature-associated increases in metabolic rate are accompanied by an increase in discontinuous gas exchange cycle frequency and either a decline (Buck and Keister, 1955; Quinlan and Lighton, 1999; Vogt and Appel, 2000; Duncan and Dickman, 2001) or no change (Lighton and Wehner, 1993; Davis et al., 1999; Chappell and Rogowitz, 2000; Klok and Chown, 2005) in Open-period volume. The increase in burst volume with increasing temperature and mass in *K. biedouwensis* is, in all probability, mostly a consequence of the effect of mass. In other species, such as tenebrionids, longicorns, and dung beetles (Lighton, 1991; Davis et al., 1999; Chappell and Rogowitz, 2000), weevils (Klok and Chown, 2005), and termites (Shelton and Appel, 2001) increases in $V^{\bullet}CO_2$ with mass are largely modulated by increases in burst volume. Thus, at least this species of Mantophasmatodea has gas exchange patterns similar to those of most other insects that have been investigated.

A comparison of water loss and metabolic rates of *K. biedouwensis* in the context of the scheme presented by Zachariassen et al. (1987) (see also Addo-Bediako et al. (2001)), indicates that it is similar to many insect species from more xeric environments. Given the typically semi-arid environments in which *K. biedouwensis* is found (Klass et al., 2003) water loss rates towards the low side are not surprising. Neither is the relatively high contribution of respiratory to total water loss ($\leqslant$29%). In those insect species that have been investigated, respiratory water loss typically constitutes 3–45% of the total water loss, with species from desert environments showing the larger values (Chown, 2002). In species from dry areas, low cuticular water loss rates typically mean that there is substantial covariation between total water loss and metabolic rate,

independent of the effects of body mass (Addo-Bediako et al., 2001), suggesting that respiratory water loss should make a significant contribution to total water loss (Zachariassen, 1991). In *K. biedouwensis* this appears to be the case. Nonetheless, *K. biedouwensis* diapauses in the egg during the drier, hotter summer of the winter rainfall area of the Western Cape Province where it occurs, and it is typically active only in winter and spring when conditions are far wetter. The microhabitat occupied by these insects (base of tussock vegetation) does not appear to confer any special temperature or humidity advantages, but rather probably serves as a daytime refuge against predators of this nocturnal species.

The present investigation of cuticular and total water loss rates also revealed that both measures are significantly repeatable, although the intraclass correlation coefficient for cuticular water loss rate was relatively low. Given the regression approach that is used to estimate cuticular water loss rate (Gibbs and Johnson, 2004), that the estimates were made by extrapolating water loss rate beyond the available metabolic rate data, and the small sample size for the repeatability investigations, substantial within-individual variability of estimates is not only expected, but should also be greater than variability expected from more direct measurements of water loss rate such as those provided by the infra red gas analyzer. Nonetheless, significant repeatability of estimated cuticular water loss rate provides evidence that the Gibbs and Johnson (2004) technique is useful for obtaining an approximation of cuticular water loss rate, in the absence of a closed period in the gas exchange cycle, and without the necessity of manipulative experiments (see Lighton et al., 2004). However, in cases where among-population or interspecific analyses of water losses via different routes are to be made to test hypotheses of adaptation (reviewed in Chown, 2002), the initial estimates should be verified using manipulative experiments that either make fewer assumptions, or where the consequences of making those assumptions can be more readily verified.

In conclusion, this study has provided the first physiological information on a species of Mantophasmatodea, demonstrating that it is similar to many other insect species (especially to most Polyneoptera in terms of its gas exchange characteristics), and that in terms of water loss it is a typically xeric species. This work has shown that the estimates of cuticular water loss provided by the Gibbs and Johnson (2004) technique are repeatable, and therefore that the method provides a useful first step in gauging cuticular and respiratory water loss rates in insects that do not show discontinuous gas exchange involving full closure of the spiracles.

Acknowledgments

This work was supported by Grant FA2004032000006 from the National Research Foundation to S.L.C. and by Grant ZA1308 from WWFSA and a University of Cape Town Block grant to M.D.P.

References

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2001. Revisiting water loss in insects: a large scale view. Journal of Insect Physiology 47, 1377–1388.

Buck, J., Keister, M., 1955. Cyclic CO_2 release in diapausing *Agapema* pupae. Biological Bulletin 109, 144–163.

Chapman, R.F., 1998. The Insects. Structure and Function, Fourth ed. Cambridge University Press, Cambridge.

Chappell, M.A., Rogowitz, G.L., 2000. Mass, temperature and metabolic effects on discontinuous gas exchange cycles in *Eucalyptus*-boring beetles (Coleoptera: Cerambycidae). Journal of Experimental Biology 203, 3809–3820.

Chown, S.L., 2002. Respiratory water loss in insects. Comparative Biochemistry and Physiology A 133, 791–804.

Chown, S.L., Nicolson, S.W., 2004. Insect Physiological Ecology. Mechanisms and Patterns. Oxford University Press, Oxford.

Chown, S.L., Addo-Bediako, A., Gaston, K.J., 2002. Physiological variation in insects: large-scale patterns and their implications. Comparative Biochemistry and Physiology B 131, 587–602.

Chown, S.L., Gaston, K.J., Robinson, D., 2004a. Macrophysiology: Large-scale patterns in physiological traits and their ecological implications. Functional Ecology 18, 159–167.

Chown, S.L., Sinclair, B.J., Leinaas, H.P., Gaston, K.J., 2004b. Hemispheric asymmetries in biodiversity—a serious matter for ecology. PLoS Biology 2, e406, 1701–1707.

Dallai, R., Frati, F., Lupetti, P., Adis, J., 2003. Sperm ultrastructure of *Mantophasma zephyra* (Insecta, Mantophasmatodea). Zoomorphology 122, 67–76.

Davis, A.L.V., Chown, S.L., Scholtz, C.H., 1999. Discontinuous gas-exchange cycles in *Scarabaeus* dung beetles (Coleoptera: Scarabaeidae): Mass-scaling and temperature dependence. Physiological and Biochemical Zoology 72, 555–565.

Dodds, P.S., Rothman, D.H., Weitz, J.S., 2001. Re-examination of the "3/4-law" of metabolism. Journal of Theoretical Biology 209, 9–27.

Duncan, F.D., Dickman, C.R., 2001. Respiratory patterns and metabolism in tenebrionid and carabid beetles from the Simpson Desert, Australia. Oecologia 129, 509–517.

Engel, M.S., Grimaldi, D.A., 2004. A new rock crawler in Baltic amber, with comments on the order (Mantophasmatodea: Mantophasmatidae). American Museum Novitates 3431, 1–11.

Gäde, G., Marco, H.G., Simek, P., Marais, E., 2005. The newly discovered insect order Mantophasmatodea contains a novel member of the adipokinetic hormone family of peptides. Biochemical and Biophysical Research Communications 330, 598–603.

Gibbs, A.G., Johnson, R.A., 2004. The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. Journal of Experimental Biology 207, 3477–3482.

Grimaldi, D., Engel, M.S., 2005. Evolution of the Insects. Cambridge University Press, Cambridge.

Gullan, P.J., Cranston, P.S., 2005. The Insects. An Outline of Entomology, Third ed. Blackwell Publishing, Oxford.

Keister, M., Buck, J., 1964. Some endogenous and exogenous effects on rate of respiration. In: Rockstein, M. (Ed.), Physiology of Insecta, vol. 3. Academic Press, New York, pp. 617–658.

Klass, K.-D., 2002. Mantophasmatodea: a new insect order?: response. Science 297, 731a.

Klass, K.-D., Zompro, O., Kristensen, N.P., Adis, J., 2002. Mantophasmatodea: a new insect order with extant members in the Afrotropics. Science 296, 1456–1459.

Klass, K.-D., Picker, M.D., Damgaard, J., van Noort, S., Tojo, K., 2003. The taxonomy, genitalic morphology, and phylogenetic relationships of Southern African mantophasmatodea (Insecta). Entomologische Abhandlungen 61, 3–67.

Klok, C.J., Chown, S.L., 2005. Temperature- and body mass-related variation in cyclic gas exchange characteristics and metabolic rate of seven weevil species: broader implications. Journal of Insect Physiology 51, 789–801.

Kozłowski, J., Konarzewski, M., Gawelczyk, A.T., 2003. Cell size as a link between noncoding DNA and metabolic rate scaling. Proceedings of the National Academy of Sciences of the USA 100, 14080–14085.

Krebs, C.J., 1999. Ecological Methodology, Second ed. Benjamin/Cummings, Menlo Park, CA.

Lessells, C.M., Boag, P.T., 1987. Unrepeatable repeatabilities: a common mistake. Auk 104, 116–121.

Lighton, J.R.B., 1989. Individual and whole-colony respiration in an African formicine ant. Functional Ecology 3, 523–530.

Lighton, J.R.B., 1991. Ventilation in Namib desert tenebrionid beetles: mass scaling and evidence of a novel quantized flutter-phase. Journal of Experimental Biology 159, 249–268.

Lighton, J.R.B., 1996. Discontinuous gas exchange in insects. Annual Review of Entomology 41, 309–324.

Lighton, J.R.B., 1998. Notes from underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods. American Zoologist 38, 483–491.

Lighton, J.R.B., Wehner, R., 1993. Ventilation and respiratory metabolism in the thermophilic desert ant, *Cataglyphis bicolor* (Hymenoptera, Formicidae). Journal of Comparative Physiology B 163, 11–17.

Lighton, J.R.B., Schilman, P.E., Holway, D.A., 2004. The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. Journal of Experimental Biology 207, 4463–4471.

Machida, R., Tojo, K., Tsutsumi, T., Uchifune, T., Klass, K-D., Picker, M.D., Pretorius, S.L., 2004. Embryonic development of Heel-walkers: reference to some prevolutionary stages (Insecta: Mantophasmatodea). Proceedings of the Arthropodan Embryological Society of Japan 39, 31–39.

Marais, E., Chown, S.L., 2003. Repeatability of standard metabolic rate and gas exchange characteristics in a highly variable cockroach, *Perisphaeria* sp. Journal of Experimental Biology 206, 4565–4574.

Marais, E., Klok, C.J., Terblanche, J.S., Chown, S.L., 2005. Insect gas exchange patterns: a phylogenetic perspective. Journal of Experimental Biology 208, 4495–4507.

Nation, J.L., 2002. Insect Physiology and Biochemistry. CRC Press, Boca Raton.

Picker, M.D., Colville, J.F., Van Noort, S., 2002. Mantophasmatodea now in South Africa. Science 297, 1475.

Predel, R., Roth, S., Neupert, S., Picker, M., 2005. New insect order Mantophasmatodea: species differentiation by mass fingerprints of peptide hormones? Journal of Zoological Systematics and Evolutionary Research 43, 149–156.

Quinlan, M.C., Lighton, J.R.B., 1999. Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). Physiological Entomology 24, 293–302.

Schmidt-Nielsen, K., 1984. Scaling. Why is Animal Size So Important? Cambridge University Press, Cambridge.

Shelton, T.G., Appel, A.G., 2000. Cyclic carbon dioxide release in the dampwood termite, *Zootermopsis nevadensis* (Hagen). Comparative Biochemistry and Physiology A 126, 539–545.

Shelton, T.G., Appel, A.G., 2001. Cyclic CO_2 release in *Cryptotermes cavifrons* Banks, *Incisitermes tabogae* (Snyder) and *I. minor* (Hagen) (Isoptera: Kalotermitidae). Comparative Biochemistry and Physiology A 129, 681–693.

Terry, M.D., Whiting, M.F., 2005. Mantophasmatodea and phylogeny of the lower neopterous insects. Cladistics 21, 240–257.

Tilgner, E., 2002. Mantophasmatodea: a new insect order? Science 297, 731a.

Tojo, K., Machida, R., Klass, K.-D., Picker, M.D., 2004. Biology of South African Heel-walkers, with special reference to reproductive biology. (Insecta: Mantophasmatodea). Proceedings of the Arthropodan Embryological Society of Japan 39, 15–21.

Tsutsumi, T., Machida, R., Tojo, K., Uchifune, T., Klass, K.-D., Picker, M.D., 2004. Transmission electron microscope observations of the egg membranes of a South African Heel-walker, *Karoophasma biedouwensis* (Insecta: Mantophasmatodea). Proceedings of the Arthropodan Embryological Society of Japan 39, 23–29.

Vogt, J.T., Appel, A.G., 2000. Discontinuous gas exchange in the fire ant, *Solenopsis invicta* Buren: caste differences and temperature effects. Journal of Insect Physiology 46, 403–416.

Walker, J.A., 2003. Mantophasmatodea—a new order of insects. Bulletin of the Amateur Entomologists' Society 62, 72–78.

West, G.B., Brown, J.H., Enquist, B.J., 1999. The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science 284, 1677–1679.

West, G.B., Woodruff, W.H., Brown, J.H., 2002. Allometric scaling of metabolic rate from molecules and mitochondria to cells and mammals. Proceedings of the National Academy of Sciences of the USA 99, 2473–2478.

Zachariassen, K.E., 1991. Routes of transpiratory water loss in a dry-habitat tenebrionid beetle. Journal of Experimental Biology 157, 425–437.

Zachariassen, K.E., Anderson, J., Maloiy, G.M.O., Kamau, J.M.Z., 1987. Transpiratory water loss and metabolism of beetles from arid areas in East Africa. Comparative Biochemistry and Physiology A 86, 403–408.

Zompro, O., 2001. The Phasmatodea and *Raptophasma n. gen.*, Orthoptera *incertae sedis*, in Baltic Amber (Insecta: Orthoptera). Mitteilungen des Geologisch-Paläontologischen Institutes der Universität Hamburg 85, 229–261.

Zompro, O., Adis, J., Weitschat, W., 2002. A review of the order Mantophasmatodea (Insecta). Zoologischer Anzeiger 241, 269–279.

Zompro, O., Adis, J., Bragg, P.E., Naskrecki, P., Meakin, K., et al., 2003. A new genus and species of Mantophasmatidae (Insecta: Mantophasmatodea) from the Brandberg Massif, Namibia, with notes on behaviour. Cimbebasia 19, 13–24.